Exopolysaccharides of the Phytopathogen *Pseudomonas syringae* pv. glycinea

Exopolysaccharides (EPS) of the soybean pathogen *Pseudomonas syringae* pv. glycinea were isolated from culture filtrates and infected soybean leaves. Levan (a polyfructan with a C-2 → C-6 backbone and C-2 → C-1 branching) or acetylated alginate (a linear polyuronide of C-1 → C-4-linked mannuronic and guluronic acids) was isolated from culture filtrates when bacterial strains were grown in a semisynthetic medium containing sucrose or glucose, respectively, as the primary carbon source. Acetylated alginate was the only EPS isolated from soybean [*Glycine max* (L.) Merr.] leaves inoculated with compatible (disease-inducing) strains of *P. syringae* pv. glycinea. The acetyl content of the *P. syringae* pv. glycinea alginates varied from 3 to 14%, and the amount of guluronic acid varied from less than 1 to 20%. The *P. syringae* pv. glycinea alginates from in vitro batch cultures were of lower molecular weight and polydispersity than those from in planta cultures, and both were of lower molecular weights than alginates produced by *Pseudomonas aeruginosa*.

The exopolysaccharides (EPS) of phytopathogenic bacteria have not been characterized as extensively as the EPS of other biologically important bacteria (18). Although the role of these compounds in phytopathogenesis has not been established, it has been suggested that they may be necessary for successful parasitization of plant host tissue (25). We have characterized the structure of the EPS derived from several strains of the soybean [*Glycine max* (L.) Merr.] leaf pathogen *Pseudomonas syringae* pv. glycinea. The strains represent three physiologic races which have different host specificity with respect to soybean cultivars. Our intent was to determine whether these strains produce significantly different EPS structures. The EPS derived from culture filtrates after growth in semisynthetic media containing different carbon sources or from infected soybean leaves were characterized with respect to composition and, in most cases, molecular weight.

P. syringae pv. glycinea is reportedly capable of levan production (17), but to our knowledge this finding is based solely on mucoid growth on sucrose-containing agar medium. As this manuscript was in preparation, M. Gross (Proc. 6th Int. Conf. Plant Pathogenic Bacteria, in press) presented preliminary evidence for alginate as an EPS of *P. syringae* pv. glycinea. Other non-plant pathogenic pseudomonads (*Pseudomonas fluorescens*, *Pseudomonas mendocina*, *Pseudomonas putida*) (12, 15) and the opportunistic plant and human pathogen *Pseudomonas aeruginosa* (8) have been shown to produce alginic acid.

(A preliminary report of part of this work has been presented [S. F. Osman and W. F. Fett, Proc. 6th Int. Conf. Plant Pathogenic Bacteria, in press].)

MATERIALS AND METHODS

Bacteria. *P. syringae* pv. glycinea 2159 (race 1) (9) was obtained from the National Collection of Plant Pathogenic Bacteria, Hatching Green, England. Strain K1 (race 1) (9) was obtained from B. W. Kennedy, University of Minnesota, St. Paul, Minn. The strains of races 4 (A-29-2 and J3-20-4A) and 5 (J3-17-2) were isolated by W. Fett (9).

EPS isolation and purification. Bacteria were cultured in the semisynthetic growth medium described by Bruegger and Keen (4), with glucose or sucrose as the primary carbon source at 24 g/liter and potassium phosphate in place of yeast extract. Bacteria were grown overnight on King medium B (KB) agar (19) at 28°C. Bacteria were suspended in sterile water to an optical density at 600 nm of 1.0, and 1 ml of inoculum was added per Fernbach flask (2.8 liters) containing 1 liter of medium. Flasks were shaken (G-10 gyratory shaker; New Brunswick Scientific Co., Inc.) at 200 rpm at room temperature. Growth was monitored by monitoring the optical density at 660 nm. After reaching stationary phase, cultures were checked for purity by standard dilution plating techniques. In addition, *P. syringae* pv. glycinea cultures were examined for contamination by *P. aeruginosa* by plating on KB agar medium and incubation at 41°C (lack of colonization indicates the absence of *P. aeruginosa*).

Bacteria were removed by centrifugation (16,300 × *g*, 30 to 60 min) and subsequent filtration of the supernatant through filters (pore size, 0.45 μm). After culture filtrates were concentrated to about one-tenth the original volume by rotary evaporation under vacuum (35°C), they were dialyzed (3,500-molecular-weight-cutoff tubing) extensively against water at 4°C, and then any insoluble material was removed by centrifugation (16,300 × *g*, 30 min). EPS were purified by the procedure described by Sutherland (26). The primary purification steps consisted of acetone precipitation (2 volumes of acetone at −20°C) followed by ultracentrifugation (100,000 × *g*, 4 h) and then acetone reprecipitation.

Trifoliate soybean leaves were inoculated with *P. syringae* pv. glycinea or with water as a control by forcibly spraying the abaxial surfaces with bacterial suspensions containing about 5×10^6 CFU/ml of H_2O, until approximately 90% of the tissue had a water-soaked appearance. After 7 to 10 days, inoculated leaves were detached from the plant, weighed, and vacuum infiltrated with 30 ml of H_2O (containing 100 μg of streptomycin sulfate per ml) per g (fresh weight) of leaf tissue. After the leaves stood overnight at 4°C, the absence of viable bacterial cells was shown by dilution plating techniques. Leaves were then removed by filtration, the filtrate was centrifuged (16,300 × *g*, 20 min),

the supernatant fluid was passed through a filter (pore size, 0.45 μm), and the filtrate was lyophilyzed. The solid was dissolved in a minimum amount of water or 0.1 M NaCl and passed through a gel permeation column (ACA-202 [LKB Instruments, Inc.]; fractionation range, 1×10^3 to 15×10^3) to remove low-molecular-weight contaminants. Fractions equal to the column void volume were collected.

Alginates produced by *P. aeruginosa* were kindly supplied by A. Linker, Veterans Administration Hospital, Salt Lake City, Utah.

Analytical methods. Unless otherwise stated, all reagents were supplied by Sigma Chemical Co. Levan was a commercial preparation from *Aerobacter levanicum*. Protein content was determined by a modified Lowry method (20) with bovine plasma albumin as the standard (Bio-Rad Laboratories). Carbohydrate content was determined by reaction with phenol-sulfuric acid (7) with glucose as the standard, and uronic acid content was determined by reaction with *m*-hydroxy biphenyl (3) with D-mannurono-6,3-lactone as the standard. Acetyl content was determined by reaction with hydroxylamine hydrochloride (21) with glucose pentaacetate as the standard. The thiobarbituric acid method was used for determining 3-deoxy-D-*manno*-2-octulosonic acid (KDO) (22), and the cysteine-H_2SO_4 method was used for heptose (31), with commercial KDO and sedoheptulose anhydride monohydrate as standards, respectively.

Nuclear magnetic resonance (NMR). Proton Fourier transformed spectra were obtained on samples dissolved in ^2H_2O (5 mg/ml) at 400 MHz on a JEOL GX-400 MHz instrument with an acquisition time of 2 s, a pulse delay of 2 s, and a pulse width of 6 μs. The probe was maintained at 50°C, and typically 500 scans were accumulated.

Carbon (^{13}C) spectra were obtained on the same instrument at 100 MHz in the completely proton-decoupled mode at sample concentrations of ~100 mg/ml of H_2O. Acquisition time was 0.6 s, pulse delay time was 4 s, and pulse width was 0.7 μs. Typically, 1,300 to 1,500 scans were accumulated, and probe temperature was 25°C.

For both proton and carbon spectra, sodium 3-(trimethylsilyl)-propionate-d_4 was the internal standard.

Gas chromatography-mass spectrometry (GC-MS). A Hewlett-Packard 5995B gas chromatograph-mass spectrometer fitted with a 20-m OV-101 capillary column was used for all GC-MS analyses. The column was temperature programmed from 100 to 250°C at 4°C/min.

EPS hydrolysis and monosaccharide characterization. Fructans (2 mg) were taken up in 1 ml of 1 M oxalic acid and hydrolyzed at 70°C for 1.5 h. After cooling, the oxalic acid was neutralized with $CaCO_3$, the precipitate was removed by centrifugation, and the supernatant was concentrated to dryness under a stream of nitrogen in preparation for derivatization. Reduced alginates (2 mg) were dissolved in 1 ml of 1 N H_2SO_4 and heated at 100°C for 1.5 h. After cooling, the solution was neutralized with $BaCO_3$ and centrifuged, and the supernatant was concentrated as described above.

Sugars were characterized as the aldononitrile derivatives (30) by using capillary gas chromatography (25-m OV-101 column programmed from 100 to 250°C at 4°C/min).

Reduction of polyuronides. An aqueous solution of uronide (5 mg in 1 ml) was added to a vigorously stirred solution of 100 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in 10 ml of H_2O (pH 4.75). The pH was maintained at 4.75 with the addition of 0.1 N HCl by using a pH stat (Radiometer A/S). After 2 h (at which time the reaction of uronide with carbodiimide was virtually complete), the pH of the solution was adjusted to 7 and added to a 2 M $NaBH_4$ solution (25 ml). After 1.5 h, the solution was neutralized with Dowex 50 (H^+ form), extensively dialyzed against water, and lyophilized. Reduction, as determined by uronide analysis, was generally between 80 to 90% complete.

Permethylation of polysaccharides. A modification of the procedure described by Bjorndal, et al. (2) was used. To 5 mg of sample dissolved in 5 ml of dimethyl sulfoxide (Aldrich Chemical Co., Inc.), 5 ml of potassium methylsulphinyl carbanion (~1.6 M, prepared by the addition of 5 g of 50% oil dispersion of potassium hydride to 5 ml of dimethyl sulfoxide) was added. The solution was sonicated for 30 min, stirred for an additional 3 h, and then cooled to 15°C with water. Methyliodide (1 ml) was added, and the mixture was stirred for 30 min. The solution was then added to 3 volumes of ice water, dialyzed overnight, and lyophilyzed.

Preparation of permethylated alditol acetates. The crude permethylated polysaccharide was hydrolyzed in two stages; the sample was taken up in 90% aqueous formic acid (1 ml) and maintained at 100°C for 1 h (in a screw-cap vial) after which the formic acid was removed under a stream of nitrogen at room temperature and the residue was taken up in 1 ml of 0.25 N H_2SO_4. After 16 h at 95°C, the sample was cooled, diluted with 2 volumes of water, and neutralized with $BaCO_3$; the precipitate was removed by filtration through a medium glass sintered funnel, and the filtrate was concentrated to 1 ml.

The permethylated monosaccharides were reduced by adding 0.25 mg of $NaBH_4$ to the 1-ml solution, and after 2 h at room temperature, the excess $NaBH_4$ was destroyed by neutralizing the solution with 2 M acetic acid. The solution was concentrated under a stream of nitrogen at room temperature and then repeatedly dissolved in 5% methanolic acetic acid and concentrated five times. The residue was acetylated by adding 100 μl of dry pyridine followed by 50 μl of acetic anhydride and heating for 30 min at 70°C. The solution was concentrated under a stream of nitrogen and dissolved in 50 μl of chloroform.

Molecular weight determination. A gel permeation–high-pressure liquid chromatography method (11) was used to determine molecular weight parameters, with the following modifications: mobile phase, 0.1 M NaCl; and column, μBondagel-E 1000 (30 by 0.39 cm: Waters Associates, Inc.) which exhibits universal calibration for radius of gyration. The column was calibrated for radii of gyration ranging between 38.9 and 2 nm, by using a combination of broad dextran standards and narrow pullulan standards. The upper exclusion limit, as determined with narrow polystyrene standards (32) is 2×10^6 or a radius of gyration of about 70 nm. Full details of the calibration procedure will be given elsewhere. All *P. syringae* pv. glycinea in vitro alginates were passed through an ACA-202 gel filtration column before determination of molecular weight, as described above.

RESULTS

EPS from culture medium containing sucrose. Four strains of *P. syringae* pv. glycinea representing two physiologic races were cultured on semisynthetic medium containing sucrose as the primary carbon source. Most of the EPS pelleted during high-speed centrifugation (100,000 × *g*, 4 h), giving large clear pellets, and residual EPS were recovered from the supernatant by acetone precipitation. Typical yields of sedimented EPS were 287 and 80 mg/liter for race 1 strains 2159 and K1, respectively, and 333 and 253 mg/liter for race 4 strains A-29-2 and J3-20-4A, respectively. Typical yields of EPS precipitated from the supernatant fluids were

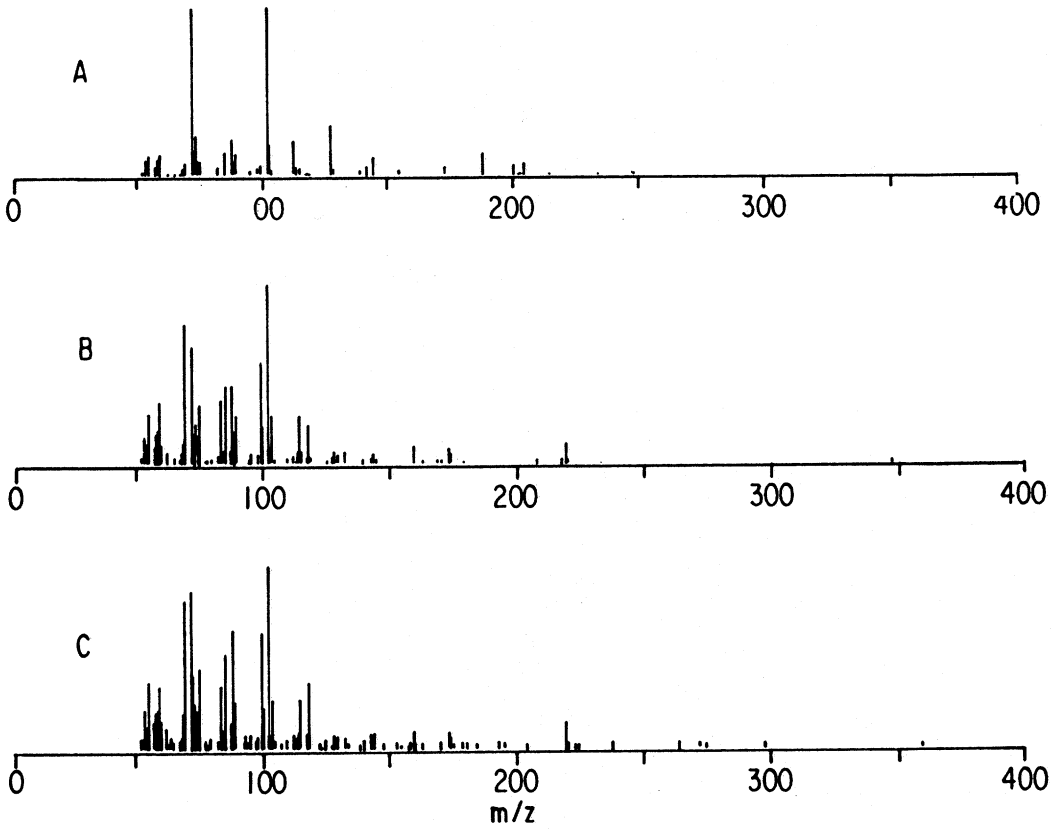

FIG. 1. Mass spectra of permethylated acetates of the major anomer of (A) inulin (C-2 → C-1), (B) *P. syringae* pv. glycinea 2159 EPS isolated from sucrose-containing medium, and (C) levan (C-2 → C-6).

25, 8, 22, and 11 mg/liter for strains 2159, K1, A-29-2, and J3-20-4A, respectively. Protein content ranged from 1 to 5% by weight, and no nucleic acid was detectable in any of the preparations. Hydrolysis in strong acid (1 N H_2SO_4 or 2 N trifluoroacetic acid) resulted in decomposition, and no monosaccharides could be detected. Hydrolysis of the sedimented or nonsedimented EPS with 1 M oxalic acid at 70°C for 90 min yielded fructose, thus suggesting that the polysaccharides were levans (C-2 → C-6 fructan backbone) or inulins (C-2 → C-1 fructan). To distinguish between these two possibilities, the polysaccharides were permethylated, hydrolyzed, and then acetylated. The GC-MS analysis (Fig. 1) conclusively proved that the *P. syringae* pv. glycinea fructans were of the levan type.

TABLE 1. Permethylated alditol acetates from *P. syringae* pv. glycinea fructans

Strain and retention time (min)[a]	Relative amt	Compound (alditol)	Substitution (C-2 → *x*)
A-29-2			
6.36	0.34	1,3,4,6-tetramethyl 2,5-diacetyl	Unsubstituted
9.60	1	1,3,4-trimethyl 2,5,6-triacetyl	*x* = C-1 or C-6
10.84	0.35	3,4-dimethyl 1,2,5,6-tetraacetyl	*x* = C-1 and C-6
2159			
6.38	0.13	1,3,4,6 tetramethyl 2,5-diacetyl	Unsubstituted
9.54	1	1,3,5-trimethyl 2,5,6-triacetyl	*x* = C-1 or C-6
10.80	0.13	3,4-dimethyl 1,2,5,6-tetraacetyl	*x* = C-1 and C-6

[a] See Materials and Methods for gas chromatographic conditions.

GC-MS analysis of the permethylated alditol acetates indicated that the levans sedimented by high-speed centrifugation of the two strains (representing two different races) of *P. syringae* pv. glycinea analyzed had different degrees of C-2 → C-1 branching (Table 1).

EPS from inoculated leaves or culture medium containing glucose. When *P. syringae* pv. glycinea 2159 and A-29-2 were cultured on medium containing glucose as the primary carbon source, purified EPS high in uronic acid (50 to 75% by the colorimetric assay) was isolated. This EPS, unlike the levans, did not pellet during the high-speed-centrifugation (100,000 × *g*, 4 h) purification step. However, small brown pellets were obtained that gave an opalescent solution in water after gentle heating, which suggested the presence of bacterial lipopolysaccharide most likely caused by cell autolysis. This was further indicated by positive colorimetric assays for heptose (0.7 to 6.0%) and KDO (1.3 to 1.8%). Protein was also present in the pelleted material at high levels (28 to 36%). EPS were subsequently recovered from the supernatant fluid by acetone reprecipitation. Average yields of purified EPS for three experiments were 40 mg/liter for strain A-29-2 and 156 mg/liter for strain 2159. Protein content ranged from 3 to 7% by weight, and no nucleic acid was present.

Similar amounts of crude material (from 20 to 60 mg/g

[fresh weight] of leaf) were obtained after lyophilization of water extracts from leaves of compatible soybean cultivar-*P. syringae* pv. glycinea interactions (cv. Harosoy versus strains A-29-2 and J3-17-2 and cv. Acme versus strains A-29-2 and 2159) showing extensive water-soaked-lesion formation, from incompatible interactions (cv. Harosoy versus strain 2159 and cv. Acme versus strain J3-17-2) showing slight chlorosis only, and from water-sprayed control leaves. However, the amounts of material which eluted in the first fraction collected after the void volume from the gel permeation column ranged from 5.4 to 7.9 mg/g (fresh weight) of leaf, with a uronic acid content of 32 to 50% (by colorimetric assay), for the compatible interactions but ranged only from 0 to 0.2 mg/g (fresh weight) of leaf, with a uronic acid content of less than 4% (by colorimetric assay), for the incompatible interactions and water-sprayed controls. Later column fractions from either compatible or incompatible interactions did not contain uronic acid. EPS from the compatible interactions for strains A-29-2 and 2159 had 2 to 4% protein by colorimetric assay and did not contain nucleic acid. EPS from the compatible strain J3-17-2–cv. Harosoy interaction had 25% protein and no nucleic acid. Alginate from *P. aeruginosa* Q60 gave a value of 40% uronic acid by colorimetric assay.

Attempts to acid hydrolyze these in vitro and in planta polysaccharides under a variety of conditions were unsuccessful in that appreciable decomposition occurred. The polyuronides were reduced by the method of Taylor and Conrad (28), and mannose and gulose were identified as the monosaccharide components by GC-MS analysis of a hydrolysate of the reduced polymer. To confirm that mannose and gulose were not present in the unreduced polymer, the reduction was carried out with sodium borodeuteride as the reducing agent. No undeuterated gulose or mannose could be detected by GC-MS analysis, indicating that the polyuronides were alginates. High-resolution ^{13}C-NMR (decoupled spectra) indicated that the alginates were acetylated (21.5 ppm, singlet), and this was confirmed by colorimetric assay. The guluronic acid content and degree of acetylation are summarized in Table 2. Similar acetylated alginates (but not levan) were produced by *P. syringae* pv. glycinea strains in planta on compatible soybean cultivars (Table 2). The presence of a signal in the ^{1}H-NMR spectrum of alginates at 4.46 ppm is indicative of consecutive guluronic acids in the polymer (13). In the analysis of three *P. syringae* pv. glycinea samples, guluronic acid blocks were detected in two samples (strain A-29-2 in vitro preparation B and strain 2159 in vitro preparation A), but none was detected in the third sample (strain A-29-2 cultured in cv. Acme). Permethylation of the reduced alginates of strains 2159 and A-29-2 obtained from in vitro cultures followed by hydrolysis and acetylation yielded 1,4,5-triacetyl 2,3,6-trimethyl hexose as the only product confirming the C-1 → C-4 backbone structure.

TABLE 2. Composition of *P. syringae* pv. glycinea alginates

Strain (prepn)[a]	% Guluronic acid[b] (NMR[c])	% Acetate[d]
A-29-2		
in vitro		
A	7 (21)	7
B	20	ND[e]
C	14 (35)	ND
in planta (cv. Harosoy)	9	14
in planta (cv. Acme)	<1	12
2159		
in vitro		
A	16 (19)	7
B	12	ND
C	13	3
in planta (cv. Acme)	<1	12
J3-17-2 in planta (cv. Harosoy)	9	9

[a] In vitro cultures were cultured on glucose-containing medium on three different dates (A, B, C).
[b] Determined by a modification of the method described by Vadas et al. (29). % mannuronic acid = 100 − % guluronic acid.
[c] Determined by the ratio of area of C-4 in mannuronic acid to C-4 in guluronic acid (14).
[d] Determined by the colorimetric method of McComb and McCready (21).
[e] ND, Not determined.

TABLE 3. Molecular size and weight parameters for *Pseudomonas* alginates

Strain (prepn)	Rg_n[a] (SD)	DP_n[b] (SD)	$M_n(10^{-3})$[c]	M_w/M_n[d]
P. aeruginosa Q52	450 (±4)	267.4 (±2.4)	47.1	1.07
P. syringae pv. glycinea A-29-2				
A[e]	31.3 (±0.3)	21.7 (±0.2)	3.8	1.05
B[e]	42.0 (±0.8)	29.7 (±0.6)	5.2	1.05
Harosoy[f]	182 (±9)	126 (±7)	22.2	1.34
Acme[f]	163 (±7)	113 (±7)	19.9	1.45
2159 (C[e])	31.8 (±0.8)	22.0 (±0.6)	3.9	1.03

[a] Number average radius of gyration.
[b] Number average degree of polymerization.
[c] Number average molecular weight.
[d] Ratio of number average molecular weight to weight average molecular weight.
[e] Cultured in a semisynthetic culture medium containing glucose as the primary carbon source.
[f] Cultured in soybean leaves (compatible reaction).

Molecular weight and size parameters for the bacterial alginates are summarized in Table 3. Values for the in vitro *P. syringae* pv. glycinea preparations are for the first fractions collected after the gel filtration column void volume. Later uronic acid-containing fractions gave similar values. It is assumed that these polymers behave like helical rods (24) and, therefore, that the degree of polymerization (DP) can be determined by the following formula (10): $DP = Rg\sqrt{12}/h$ where Rg is radius of gyration and h is the virtual bond length of an alginate monomer residue in the polymer, which can be taken as 0.5 nm (24).

DISCUSSION

Depending on the primary carbon source, the phytopathogen *P. syringae* pv. glycinea elaborates different EPS structures. Levan (C-2 → C-6 fructan backbone) is produced when sucrose is the primary carbon source. This polysaccharide, which is synthesized exocellularly by the action of levansucrase on sucrose (18), is elaborated by several bacterial genera including *Acetobacter*, *Bacillus*, *Enterobacter*, *Pseudomonas*,, *Streptococcus*, and *Xanthomonas* (18, 27). The ability to produce levan is used as a taxonomic criterion to differentiate *Pseudomonas* species (23). Typically, mucoid growth on sucrose-containing agar medium is used as the sole criterion of levan production (17). To our knowledge there is no chemical evidence for levan production by the approximately 40 *Pseudomonas*

syringae pathovars (23) except for aptata, morsprunorum, phaseolicola, syringae, and tabaci (1, 5). In this study we characterized the EPS of several strains of *P. syringae* pv. glycinea cultured on sucrose-containing medium, by suitable chemical analysis, as branched (at carbon 1) levans. The degrees of branching for the two strains analyzed (A-29-2 and 2159) were significantly different. Since these strains represent different physiologic races, it will be of interest to examine additional strains to determine whether there is a correlation between bacterial race and degree of branching of the levans.

Since levan can only be produced when sucrose is available, it was of interest to determine the structure of the EPS produced, if any, when glucose was substituted for sucrose in the culture medium. With glucose as the primary carbon source, the only EPS detected was the polyuronide alginic acid. Although most commonly found as structural polysaccharides in brown seaweeds, alginic acid (partially acetylated) is elaborated as an EPS from *P. aeruginosa* and *Azotobacter vinelandii* (16). The *P. syringae* pv. glycinea alginates were very water soluble even at pH 1 and gave relatively nonviscous solutions in comparison to *P. aeruginosa* alginates. We observed significant variability in the ratios of guluronic to mannuronic acid in alginates from the same strain isolated from in vitro batch cultures at different dates. The guluronic acid concentrations of the alginates formed in vitro, however, were in most cases higher than the concentration found in the alginate produced by strains 2159 and A-29-2 in planta. The in planta samples also had a higher degree of acetylation than the in vitro samples. The value for percent acetate given in Table 2 for alginate of the strain J3-17-2–cv. Harosoy interaction is lower than the actual value due to the significant amount of protein (25% [wt/wt]) in these preparations. The higher degree of acetylation is consistent with the hypothesis that acetylation occurs on the mannuronic acid residues (6), since the in planta samples were higher in mannuronic acid than the in vitro samples. Similar to *Azotobacter vinelandii* (16), the *P. syringae* pv. glycinea strains cultured in vitro contained blocks of guluronic acid. The presence of guluronic acid blocks has been independently confirmed by I. W. Sutherland for an alginate preparation produced in vitro by *P. syringae* pv. glycinea 2159, by using a guluronate-specific lyase (personal communication). Our failure to detect guluronic acid blocks in the one alginate produced in planta that was analyzed may have been due to the very low concentration of guluronic acid present in the polymer.

Gross (in press) has reported alginate production in vitro for *P. syringae* pv. phaseolicola, *P. syringae* pv. glycinea (races 2, 4, and 6), *P. syringae* pv. tomato, *P. syringae* pv. pisi, and *P. syringae* pv. lachrymans. We have also found that several pathovars of *P. syringae* in addition to *P. syringae* pv. glycinea produce alginate (Osman and Fett, in press; manuscript in preparation). Similar to our results for *P. syringae* pv. glycinea in planta, *P. syringae* pv. phaseolicola produced alginate alone in infected host leaves, but Gross reported that both levan and alginate were isolated from bacterial ooze on infected stems (in press). The possibility that levans are produced by these bacteria in host leaves but are subsequently hydrolyzed to lower-molecular-weight fructans by plant or bacterial β-fructosidases or both has not been ruled out.

The high degree of water solubility of the *P. syringae* pv. glycinea alginates made it possible to obtain ^{13}C-NMR spectra on these compounds in both the acetylated and deacetylated forms (deacetylation was readily accomplished by adjusting an aqueous solution of alginate to pH 10.5 and, after 24 h at room temperature, neutralizing, dialyzing, and lyophilizing the solution). Although we were not able to determine whether sequences of alternating guluronic and mannuronic acid were present by NMR techniques (14) because of insufficient resolution of the critical peaks in the spectra, we were able to estimate the guluronic-to-mannuronic-acid ratio by ^{13}C-NMR. In these three cases in which this was done, ^{13}C-NMR gave consistantly higher guluronic acid values than our gas chromatography analytical procedure. We have recent evidence that the reduction method via the carbodiimide adduct, which is a step in the procedure for guluronic- and mannuronic-acid determination in alginic acid (29), may be responsible for this discrepancy.

Molecular weight parameters were determined by gel permeation–high-pressure liquid chromatography. By this method the *P. syringae* pv. glycinea alginates isolated from in vitro cultures were considerably lower in molecular size and weight than *P. aeruginosa* alginates, which is consistent with the lower viscosity observed for the *P. syringae* pv. glycinea alginate solutions. Alginates produced by *P. syringae* pv. glycinea in planta were higher in molecular weight and in polydispersity (M_w/M_n in Table 3) than the corresponding alginates produced in vitro. The low molecular weights of alginates produced in vitro by *P. syringae* pv. glycinea strains may have been due to production and secretion of alginases by these bacteria.

In conclusion, the chemical composition of *P. syringae* pv. glycinea EPS is highly dependent on the primary carbon source and environmental factors which obscure any subtle structural difference in EPS from different bacterial strains. The observed differences in the degree of branching in levan isolated from two physiologic races of *P. syringae* pv. glycinea require further experimentation. However, alginate, and not levan, appears to be the EPS of biological interest since only alginate was detected in planta.

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